GAMETOGENESIS IN ALLOMYCES ARBUSCULA

BY

WINSLOW ROPER HATCH

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Gametogenesis in Allomyces arbuscula.[1]

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WINSLOW R. HATCH.

(*The Johns Hopkins University.*)

With thirty-three Figures in the Text.

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I. HISTORICAL INTRODUCTION.

THE genus *Allomyces* was established in 1911 by Butler (3) to house a new member of the Blastocladiaceae which he collected in India. In this plant, which he named *A. arbuscula*, no evidence of sexuality was seen. The next year the same species was found in Ithaca, N.Y. by Barrett (2), who also described it as lacking in means of sexual reproduction. In 1922 Coker and Grant (5) isolated the same fungus at Chapel Hill, N.C., and since no sexual reproductive structures were discovered they were disposed to interpret certain resting sporangia as 'unfertilized eggs' that developed parthenogenetically. This fungus has been isolated from Mississippi soil by Harvey; and Lugg (12), 1929, studied material supplied by Harvey. A species of *Allomyces* has also been reported from the Philippines by Weston (18) and still another species, *A. moniliformis*, has been described from Smith Island, N.C. by Coker and Braxton (4). It was not until 1929, however, that sexuality was discovered in the genus. In that year Kniep (11) was able to distinguish gametangia, and to demonstrate the fusion of gametes in *A. javanicus*, a new species, which he discovered in Java.

[1] Botanical contribution from The Johns Hopkins University, No. 129.

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Although the first published account of sexuality in *Allomyces*, that of Kniep, appeared in 1929, Weston (18) ten years before had observed in his Philippine species that among the 'zoosporangia' there was a regular alternation of large, ovoid to spherical 'sporangia', with small, cylindric to barrel-shaped 'sporangia', 'and that the larger sporangia were greyish-protoplasmic in colour, the small, barrel-shaped ones orange'. He made drawings of these 'sporangia' and 'zoospores', and at the top of one sheet of drawings he had written, 'Are these gametangia and gametes?' This question remained unanswered until Kniep published his article on the sexuality of *A. javanicus*.

Kniep's discovery of sexuality in *Allomyces* was epochal not only because it clearly demonstrated, for the first time, the occurrence of sexuality in the Blastocladiaceae, but because it was also the first case of heterogamous, planogametic conjugation known to exist among the Phycomycetes. In *A. javanicus* the male and female gametangia appear at the hyphal tips, the male commonly being terminal. The smaller, male gametangium is orange to brick-red; the female is grey or colourless. Both are of the same ellipsoidal shape. The male and female gametes are alike in that each is ellipsoidal and uniciliate. They are, however, different in size, the male gamete being about half the size of the female. These uniciliate hetero-gametes conjugate to form biciliate plano-zygotes. The zygotes germinate in three to four hours, forming new plants. Kniep was unable, in living material, to follow the process of conjugation in its entirety, but he was able to demonstrate it cytologically. He found a complete series in which he could trace cytoplasmic and nuclear fusion.

The first published confirmation of Kniep's observations was made in 1933 when sexuality was described in *A. arbuscula* Hatch (10). In *A. arbuscula* the male and female gametangia are normally paired. They appear at the hyphal tips, the female terminal. The male and female gametangia differ in colour, shape, and size, the male gametangia being salmon-pink, barrel-shaped, and of smaller size, the female ashen grey, more nearly spherical, and notably larger. The gametes differ in colour, size, and motion. The male is of a brassy colour, the female grey. The male is one-half to one-third the size of the female, and shows a more active, darting motion than the female. Both are of the same spherical to subglobose shape. The conjugation of living gametes was described in detail.

Kneip considered *A. javanicus* of specific rank because it was the only species of *Allomyces* then known to be sexual. Although he used this distinction alone to separate it from *A. arbuscula*, *A. javanicus* still stands as a distinct species, even though sexuality has been discovered in *A. arbuscula*, because in *A. javanicus* the male gametangium is commonly terminal on the hypha, whereas in *A. arbuscula* it is the female that is regularly so. In this connexion it is interesting to note that Kniep isolated,

in addition to *A. javanicus*, another species of *Allomyces* that contrasted in its sexuality with *A. javanicus*, as does *A. arbuscula*. This 'Bali' species, when re-investigated, may very well prove to be *A. arbuscula*, or a variety of this species.

The curious and altogether remarkable type of sexuality described above deserves careful analysis. To this end a study of gametogenesis in *A. arbuscula* was inaugurated.

II. Material, Cultural Methods, and Cytological Technique.

Material.

The material for this study was collected from a stream near Raleigh, N.C., July 7, 1931. Since that time it has been kept in culture in the Botanical Laboratory at the University of North Carolina, and it was from these cultures that mycelia were selected and brought to this laboratory by Prof. John N. Couch.

Cultural methods.

With the discovery of sexuality in *A. arbuscula* it became apparent that there are two types of mycelia, asexual mycelia bearing zoosporangia and resting sporangia, and sexual mycelia bearing gametangia and resting sporangia. The asexual mycelia are derived from zoospore or zygote isolations; the sexual mycelia from isolations of zoospores from resting sporangia formed on sexual or asexual mycelia. To obtain sexual mycelia the following method can be recommended:

1. Dry sterile agar cultures of either sexual or asexual mycelia at room temperature for a few weeks to several months, or dry at 40° to 45° C. for one to two hours.

2. To dried cultures add distilled water to soften agar.

3. Cut out 1 square centimetre of agar and inoculate hemp seed (4 halves of cotyledons) in aqueous culture.

4. Sexual mycelia will be ready to study or to fix in about three or four days.

This method, beside assuring the production of sexual mycelia, can be recommended because material can be stored in the dry condition indefinitely, cultures can be kept free of bacterial contamination, and the amount of inoculum can be controlled.

Cytological technique.

In the study of living material whole mycelia were used. After washing out the selected mycelia in fresh water they were either transferred to hanging drops or mounted under supported cover-slips. The change of conditions given by washing provides a strong impetus to gametogenesis, which can then be carefully followed under the high

powers of the microscope. The source of illumination, unless otherwise stated, was artificial light. Of the vital stains employed in this study Janus green and neutral red were most relied upon. Janus green was found specific for chondriosomes when used in the proper dilutions, and a technique employing neutral red was developed whereby differential staining of the vacuoles could be accomplished.

In the study of fixed material it was found both practicable and indeed advisable to use whole mycelia. A great many different fixatives and stains were tested for artifacts by constant comparison with the conditions obtaining in the living material. In the nuclear study the Feulgen reaction was found to be most adequate. In the investigation of the cytoplasmic inclusions several methods recommended themselves. For the lipoid granules an osmic acid technique proved most satisfactory. For the chondriosomes three methods were used: Regaud's, Meves', and a combination of Benda fixation and Champy-Kull staining. Of the three the Benda-Champy-Kull combination was probably the best; but all methods were used, the evidence from one supplementing that from the others. All the types of technique mentioned above are described in Lee's 'Microtomist's Vade-Mecum', ninth edition, with the exception of an adaptation of the Feulgen reaction for chromatin, which is herewith printed in full.[1]

III. Observations on Living Material.

(a) *Morphological considerations.*

In gametogenesis an important feature is the striking colour change that occurs in the male gametangium. Since the colours are more intense in daylight all observations on this particular phenomenon were made with this type of illumination. In the hyphal tips (Fig. 1) the concentrated cytoplasm appears grey-black in colour. When the gametangia are cut off from the hypha their contents are likewise grey-black. Later, both gametangia show a distinct bulging of their lateral walls, but there is no increase in diameter in the region of the cross walls, so that a definite constriction arises between the female and the male gametangium and a lesser one between the male gametangium and the hypha proper. At this stage the male gametangium lightens in colour, taking on a yellow tint. Still later, as the female gametangium continues to increase in diameter,

[1] The Feulgen reaction for chromatin is as follows: 1. Fix in Feulgen's fixative, 20 minutes. 2. Wash in distilled water through several changes. 3. Hydrolysis: Cold 0·5 N hydrochloric acid, 10 minutes. Hot 0·5 N hydrochloric acid, 58° C., 18 minutes. Cold 0·5 N hydrochloric acid, rinse. In the hot hydrochloric acid do not let the temperature rise above 60° C. 4. Stain in fuchsin-sulphurous acid, 1 hour. Glassware (vials) must be perfectly clean. Wash in cleaning solution and rinse well before using. 5. Wash in sulphurous acid, two changes. 6. Rinse in tap water. 7. Dehydrate in alcohol. 8. Clear in xylol. 9. In xylol, dissect mycelium from the hemp seed, wash out. 10. Mount in balsam. For the formulas of fixative, stain and other solutions, see Lee's 'Microtomist's Vade-Mecum', ninth edition, page 306.

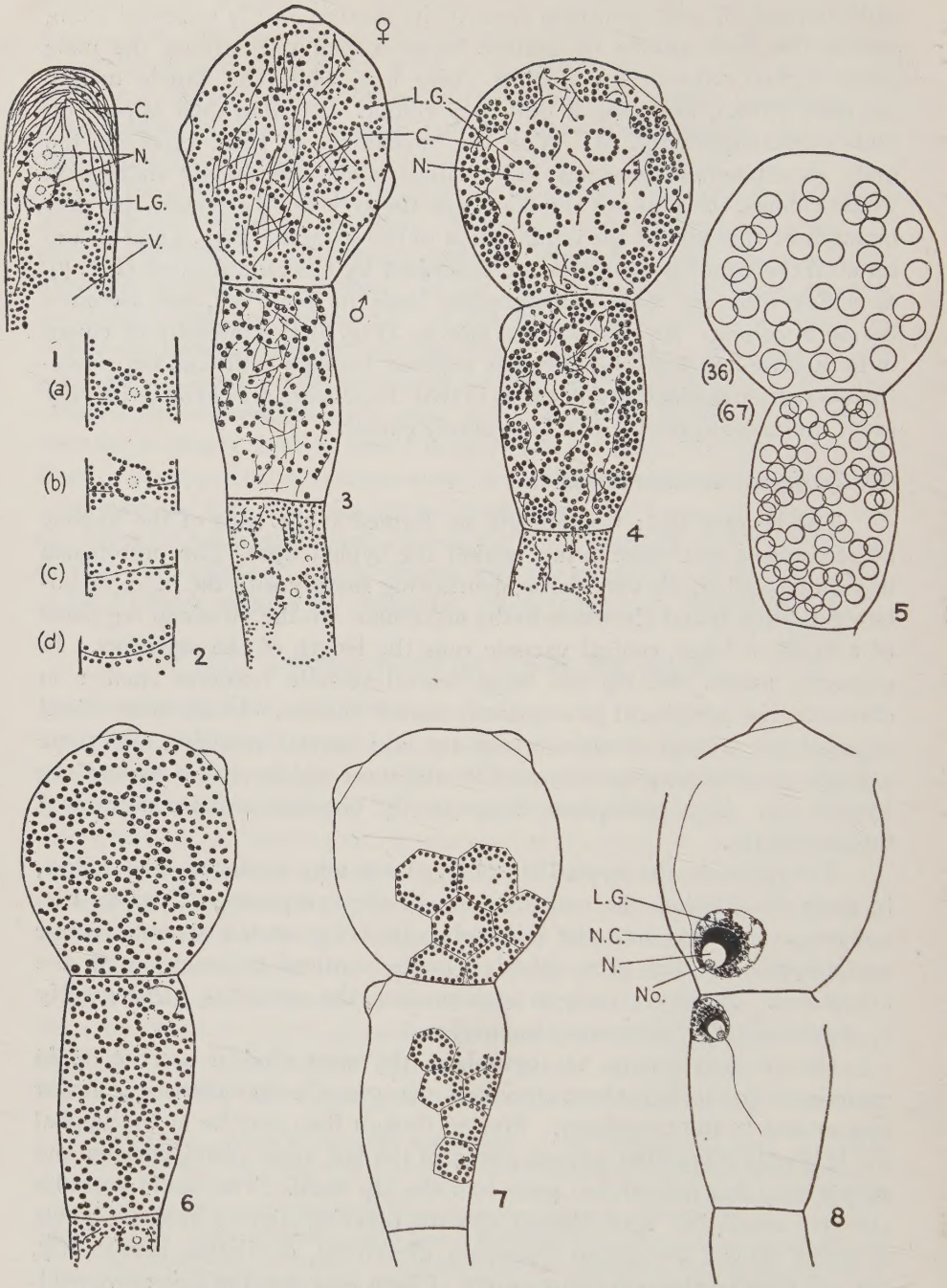
and to round off as it advances towards its mature, nearly spherical form, and as the male attains its mature barrel-shaped proportions, the male gametangium takes on a distinct rusty hue, while the female merely becomes lighter, assuming a dull grey colour. Papillae then appear on both gametangia (Fig. 3). With the appearance of these processes the male gametangium becomes light salmon-pink, the female dull grey. These colours, the pink for the male, and the grey for the female, are now maintained throughout the organization of the gametes (Figs. 4 and 6), and up until the time the gametes are delimited by cleavage furrows (Fig. 7), at which time the male gametangium loses its pink tint, and becomes brassy in colour. As the gametes emerge (Fig. 8) the intensity of colour in both male and female gametes is reduced through diffusion, but nevertheless a sharp distinction still exists in that the females are characteristically grey, the males typically of a pale brassy colour.

(b) *Cytological considerations.*

The Hyphal tips: Gametangia are formed at the ends of the hyphae by cross walls that come in just behind the hyphal tips. The protoplasm in such hyphal tips is very dense, contrasting sharply with the more vacuolate condition found elsewhere in the mycelium. In the proximal segments of a hypha a large, central vacuole runs the length of the segments. In segments nearer the tip the large central vacuole becomes smaller in diameter, the peripheral protoplasmic sheath thicker. In the most distal segment the central vacuole breaks up into several vacuoles, and these vacuoles become progressively smaller and more widely spaced toward the hyphal tip. The protoplasm consequently becomes more concentrated toward the tip.

The nuclei in the hyphal tip (Fig. 9) are very numerous, and follow, in their distribution, the concentration of the cytoplasm. They do not, however, move out into the tip itself. In living unstained material the nuclei appear as faint, grey, spherical bodies outlined by inclusions in the cytoplasm. A darker body in each nucleus, the nucleolus, helps greatly in the certain identification of the nuclei.

Of the inclusions in the cytoplasm the most obvious are the lipoid granules (Fig. 1). It is these structures that give the characteristic granular appearance to the cytoplasm. Massed though they may be in the hyphal tip, it is only a few that extend ahead of the last, most distal nucleus, and only a very few indeed that push into the tip itself. The lipoid granules are very small, but very distinct spheres, differing slightly in size. These granules show a restrained Brownian movement, oscillating persistently, but not moving through a large orbit. Either as a result of this movement, or from their inherent nature, or both, these lipoid granules aggregate about the larger, less mobile protoplasmic structures such as the nuclei,



FIGS. 1–8. Gametogenesis in living material. × 900. 1. Hyphal tip vitally stained with Janus green showing distribution of chondriosomes and lipoid granules. 2. *a*, *b*, *c*, *d*. Stages in gametangial cross-wall formation. 3. Male and female gametangia. The 'granular' stage of

and the plastids, and even line up along the chondriosomes. With artificial light these granules appear olive-grey in colour; with daylight, grey-black. They are, apparently, exactly the same type of granule as that which Guilliermond noted in *Saprolegnia* (7, 9) and *Leptomitus* (8), and which he described as 'lipoid granules'.

In among the lipoid granules, and in turn reaching ahead of them, are the chondriosomes (Fig. 1). These structures become strikingly concentrated into a weft of filaments that fits into the constricted hyphal tip. From this chondriosomal complex there depends peripherally a mantle of long chondriosomes. This mantle frays at its end, and merges gradually into the loose, peripheral arrangement of chondriosomes characteristic of the rest of the hypha. All the chondriosomes in this apical complex are filamentous, much attenuated, and very unstable in that they are capable of breaking up into short rods. The chondriosomes in all the more proximal segments of the hypha attain greater length and girth, and are so spaced that they can be readily distinguished the one from the other. Their long axes parallel the axis of the hypha. In the living, unstained condition, the chondriosomes appear light blue-grey. In a living hypha chondriosomes move sluggishly about, but whether this movement is due to cytoplasmic streaming, to the bombardment of lipoid granules in Brownian movement, or in part to their own activity, cannot be said. Vital staining with Janus green reveals no new structures, but stains chondriosomes specifically, and so helps to identify chondriosomes and chondriosomal derivatives more definitely. The chondriosomes of *A. arbuscula* have much the same appearance as those described by Guilliermond for *Leptomitus* (8) and *Saprolegnia* (7, 9).

Cytoplasmic streaming is very important in bringing about this concentration of the nuclei, the lipoid granules, and the chrondriosomes at the hyphal tip. These three constituents can be seen moving up the hypha, and can even be seen threading the pseudosepta. This movement is most apparent in the lipoid granules, which are to be observed constantly moving up along the inner face of the peripheral sheath of cytoplasm.

When gametangia are formed, two cross walls come in across just such a hyphal tip as that described above. These walls, unlike the perforate pseudosepta, are complete. They are formed by the ingrowth of two ridges that appear on the inner wall of the hypha. These ridges develop into thin diaphragms which finally close across the lumen of the hypha to

gametogenesis. Chondriosomes and lipoid granules widely scattered. 4. Male and female gametangia. The 'gamete origin' stage of gametogenesis. Nuclei displaced peripherally, outlined by lipoid granules. Chrondriosomes in the interstitial cytoplasm. 5. 'Gamete origin' stage showing relative number and size of nuclei in male and female gametangia. 6. 'Disappearance' stage. Lipoid granules scattered. Outline of nuclei lost. Chondriosomes no longer apparent. 7. Cleavage. Lipoid granules alone apparent and these displaced peripherally in the individual gametes. 8. Male and female gametes each with a nucleus, a nuclear cap, and lipoid granules. C. = chondriosomes; L.G. = lipoid granules; N. = nucleus; NO. = nucleolus: N.C. = nuclear cap; V. = vacuole.

form the two cross walls. When a nucleus lies directly in the path of one of these walls the wall apparently makes a detour around the nucleus, with the result that it is concave toward the nucleus. For stages in the formation of one of these cross walls, see Fig. 2 *a, b, c, d.* These two cross walls are formed at about the same time, but they are not initiated simultaneously. In a couplet of very young gametangia the wall cutting off the terminal cell or female gametangium is thicker than that which cuts off the subterminal cell or male gametangium. Furthermore, instances may be found in still younger couplets where the wall cutting off the male is not complete, when that of the female is quite complete.

Gametogenesis, 'granular' stage (Fig. 3).

The diffuse, granular appearance characteristic of the gametangia when they are cut off from the hypha is, of course, to be attributed to a dispersed distribution of lipoid granules. Here and there, but particularly toward the surface, the granules may clump together, but for the most part they are strung out through the cytoplasm in an irregular network. In this initial stage of gametogenesis the lipoid granules are of the same size, and show the same Brownian movement as the lipoid granules in the hyphal tip; and irrespective of whether they have been segregated in a male or a female gametangium they are of the same grey-black colour.

The chondriosomes, like the lipoid granules, are widely scattered. They, too, are more numerous peripherally where they lie scattered among the lipoid granules.

The vacuolate condition of the cytoplasm noted in the hypha persists in the gametangia; vacuoles are numerous and well scattered through the gametangia.

The nuclei throughout the 'granular' stage of gametogenesis are found rather deep in the gametangia, where the lipoid granules are so dispersed that they cannot effectively outline them. The greater concentration of the lipoid granules peripheral to the nuclei tends only to screen the nuclei. The nuclei in the granular stage are, consequently, far from apparent and can only be identified after careful study. Despite these difficulties, however, enough can be seen to show that when the two cross walls close across the hypha the nuclei of the hypha are segregated to the male and the female gametangium in about equal numbers. The number of nuclei in each gametangium is increased almost immediately by nuclear division. Prior to these nuclear divisions the nuclei are irregularly distributed throughout the gametangia. After these divisions the nuclei become very evenly spaced.

The 'granular' stage is a stage in which the full nuclear complement of the gametangium is achieved. Furthermore, it is a stage in which the nuclei become so distributed that when called upon to act as gamete

organization centres, each has an equal and equivalent sphere of influence in a cytoplasm which is as yet quite unorganized. Finally, it is the stage in which the mature shape of the gametangium is attained, and the first colour change is initiated in the male.

Gametogenesis, 'gamete origin' stage (Fig. 4).

Soon after the mature form of the gametangium has been attained emergence papillae put in their appearance. With this new morphological development, striking cytological changes are initiated within the gametangium. The nuclei, heretofore well removed from the gametangial walls, now all become displaced peripherally. The lipoid granules once widely dispersed follow the nuclei to the walls of the gametangium and line up about them. These nuclei with their sheaths of lipoid granules constitute the 'gamete origins'. At first the lipoid granules at the surface of the gametangium are too few to ensheath the nuclei completely, but their number is gradually augmented as more and more lipoid granules move out of the central cytoplasm. As soon as the lipoid granules approach the surface they scatter among the nuclei. Here their erratic movements carry them about among the nuclei and ultimately bring them in contact with a nucleus where they take up their position on the nuclear membrane. In addition to this new distribution of the lipoid granules this stage of gametogenesis has brought significant changes to the lipoid granules themselves. They are larger now, larger than they were in the 'granular' stage. They have also changed colour. This change has been most spectacular in the male gametangium where the lipoid granules have acquired a colour which is yellow in artificial light, and salmon-pink in daylight. In the female gametangium there has been no such marked colour change in the lipoid granules; they may have lightened in colour, but they are still grey (in either light).

The 'gamete origin' stage is the most favourable stage of gametogenesis in which to study the chondriosomes. At this time the chondriosomes are concentrated peripherally in the interstitial cytoplasm between the nuclei. With the concentration of the lipoid granules about the nuclei a clear view of this interstitial cytoplasm is afforded. The fine, blue-grey chondriosomes can be seen most advantageously in the male gametangium where the yellow lipoid granules present a good contrast.

Vacuolar changes apparently account for the striking peripheral displacement of nuclei and cytoplasm that distinguishes this stage. The vacuoles, scattered in the 'granular' stage, crowd toward the centre of the gametangium where they fuse into several larger vacuoles or a single, large, central vacuole. In the female gametangium this vacuolar system may occasionally be long and tortuous, but more commonly it is contracted toward the centre of the gametangium, and only pushes out a few blunt lobes into the peripheral cytoplasm. In the male the vacuoles fuse into

a more ramifying vacuolar system. This system is gathered toward the centre of the gametangium and extends from one end to the other.

Comparing the male and female gametangia at this stage of their development, it is apparent that there is just as striking a contrast in their respective nuclei as in the colour of their lipoid granules. It will be recalled that during the 'granular' stage there were numerous nuclear divisions in the young gametangia. It now appears that there must have been approximately twice as many mitoses in the male as in the female gametangium, for there are roughly twice as many nuclei in the male as in the female (Fig. 5). Furthermore, the nuclei of the male gametangium are but one-half the size of the nuclei of the female. No difficulty is experienced in studying the nuclei at this stage. Their extreme peripheral position and their definite outline makes their study easy. Even the nucleoli can be readily seen. A word ought to be said here also about the mobility of the nuclei. In this stage the nuclei are constantly changing position as a result of the jostling of the lipoid granules. This drifting of the nuclei is, however, to be noted at all stages of gametogenesis, so it is apparently of no particular significance. There are, however, other major nuclear displacements that are significant, such as that involved in their recent peripheral migration.

In the first or 'granular' stage of gametogenesis the nuclei become evenly dispersed throughout the cytoplasm of the gametangia. In this second or 'gamete origin' stage these nuclei serve as organization centres for the cytoplasmic constituents, drawing about themselves, first, a sheath of lipoid granules, then a tangle of chondriosomes.

Gametogenesis, 'disappearance' stage (Fig. 6).

Gametogenesis is often temporarily arrested in the 'gamete origin' stage. In old cultures almost all gametangia will be found to have reached this stage of development, there to await a change of conditions before completing the organization of the gametes. In practice, the only change of conditions found necessary was a change of water. This brought about a completion of gametogenesis within the hour. The first sign of renewed activity in such arrested gametangia is the crowding of the lipoid granules into the cytoplasm between the nuclei. Ultimately a heavy screen of lipoid granules is drawn over the whole surface of the gametangium, and the lipoid granules become noticeably motionless. The gametangia do not remain in this state of organization for any length of time, however, because very shortly the 'gamete origins' sink toward the centre of the gametangium. In this crowding of the 'origins' towards the centre of the gametangium the lipoid granules gradually become widely dispersed and the nuclei, once so distinct, become less and less easy to identify until ultimately all that can be identified as nuclei are the numerous clear areas appearing among

the lipoid granules. Thus the gametangia, for all their erstwhile organization, appear again superficially quite unorganized. The 'gamete origins' pass into a 'disappearance' stage.

Changes in the central vacuole apparently account for the central displacement of the 'gamete origins'. Back in the 'gamete origin' stage the central vacuole or vacuoles begin to send out peripheral processes. In the 'disappearance' stage the further conversion of the large central vacuole or vacuoles into a system of vacuolar wedges or cleavage furrows results in the displacement of the cytoplasm toward the centre of the gametangium. At the very outset of this cytoplasmic migration the nuclei abandoned their peripheral location for a more central position on or near the central vacuole. With the centrifugal progress of the vacuolar wedges the nuclei are left behind, each in a sort of cradle between the advancing wedges. The chondriosomes cannot be identified at this stage of gametogenesis.

Gametogenesis, cleavage (Fig. 7).

In living unstained material it is very difficult to follow the progress of the vacuolar wedges or cleavage furrows. In mycelia vitally stained with neutral red, however, the peripheral advance of the vacuolar cleavage furrows can be demonstrated. All through the 'disappearance' stage these cleavage furrows can be seen slowly pushing out through the peripheral cytoplasm. At their advancing tips the cleavage furrows are drawn out into thin, tortuous channels. Before the vacuolar wedges have had time to push to the wall of the gametangia the first gametes, those that occupy the centre of the gametangia, have already been cut out. A sort of shudder goes through the gametangium as these furrows come in. The nuclei during cleavage maintain a position deep in the gametangium where they abut on the central vacuolar system. As cleavage proceeds the lipoid granules line up along the cleavage furrow. Although there is considerable movement among the lipoid granules at this time it is, of course, restricted in that the granules can only move within the limits set by the cleavage furrows.

After cleavage has been accomplished the fate of the chondriosomes can at length be determined. It is apparent that they have undergone a striking metamorphosis, for they now appear as one, two, or three large blobs which closely invest the nucleus. As the cleaved origins, now gametes, further individualize themselves, these several chondriosomal blobs fuse until but one large one remains; this mass subsequently becomes applied to the nuclear membrane forming a sort of nuclear cap (Fig. 8).

The Gametes (Fig. 8).

Normally, the discharge of the gametes begins soon after cleavage has been completed, but in the interval before discharge there are incipient movements among the gametes within the gametangia. These movements are most apparent among the gametes that abut on the emergence

papillae, for among them there is a very definite crowding and pushing as they force their way into the papillae. After the bursting of the papillae of emergence by the first gametes there is a rather steady egress of gametes, but as the gametangia are gradually emptied the interval between the escape of the gametes at any one pore becomes longer. Thus, while the majority may have escaped in the first 10 minutes, a few (1 to 3 or more) will still be found in the gametangium 20 to 30 minutes later. Sometimes the two gametangia of a couplet may discharge almost simultaneously, but more often the male precedes the female in dehiscence, and this despite the fact that the male was cut off from the hypha somewhat later than the female.

The male gametes appear one at a time sometimes close behind each other but nevertheless always distinct. In making their escape they have first to push through a small pore the narrowness of which constricts them in the process. But even when its body is outside the gamete is not always free, since frequently it has trouble in disengaging its long cilium which it drags behind while pushing out of the gametangium. Indeed, four hapless gametes have been seen to tug for 14 minutes before they could free their cilia.

The female gametes are slow to escape. In some instances they are completely individualized when they push through the pore of the papilla. Once free of the gametangium, however, they are at first perfectly quiet. Then they begin to swing slowly on their axes through an arc of not more than 45°. This movement ultimately gives way to a rapid vibratory motion, and finally the gametes move off, each propelled by its single, posteriorly attached cilium. At other times the female gametes escape in blobs that are apparently undifferentiated and certainly remain quite motionless for a time. As the separate gametes gradually attain their individuality, rounding up and separating from the mass, it appears that as many as ten to twelve gametes may be contained in such blobs.

In the gametes all essential structures can be seen clearly (Fig. 8). Both male and female gametes are spherical to subglobose in shape, and although instances of biciliated gametes may be noted in some cultures, the gametes, male or female, have characteristically but a single, long, posteriorly-attached cilium. At the base of this cilium and at the point where the cilium touches the membrane there is a very small granule. Supplementing these observations on living gametes (Fig. 8) by a study of fixed gametes (Fig. 20) it appears that this granule, which marks the base of the cilium, is connected with the nucleus by two fine filaments. The nucleus in the gametes, as has been stated, is closely invested by a bulky nuclear cap; together these bodies form a conspicuous pyramidal structure the apex of which is directed toward the posterior end of the gamete. Since the nucleus is situated at the apex of this pyramid and since the compound

structure has been displaced posteriorly the nucleus is brought close to the gamete membrane just opposite the point of attachment of the cilium. A nucleolus is always to be found lodged against the nuclear membrane at the point at which the nucleus lies in closest proximity to the basal granule of the cilium. The two filaments which extend from the base of the cilium to the nucleus contact the nuclear membrane at two points, to the right and left of the nucleolus. These points mark the farthest extension of the chromatic reticulum about the nucleolus. In a motile gamete the two arms of the chromatic reticulum are being constantly stretched and relaxed by the movements of the nucleolus. At one time the nucleolus strikingly extends the nuclear membrane, then it momentarily sinks back into the nucleus before it again distends the membrane and so repeats the process. The nucleolus also experiences lateral displacements. Where this movement is initiated is not known, but certainly this part of the nucleus is always showing distortion.

When the gamete is in motion the nuclear cap and the imbedded nucleus act as a unit; any displacements of the one affect the other. About the nucleus and the nuclear cap the cytoplasm is dense, whereas in the peripheral regions of the gamete the cytoplasm is strongly vacuolate. The lipoid granules are generally to be found in the denser cytoplasmic region, and particularly in the cytoplasm that immediately invests the nucleus and nuclear cap. As the gamete changes the character of its movement, the lipoid granules slide around on the nuclear cap to new positions. When the gamete is swimming they usually become concentrated toward the anterior end of the cell. With the cessation of active movement, at least some of the lipoid granules glide back down and around the nuclear cap and even gather behind the nucleus.

When the gametes cease swarming, amoeboid movements begin, and a hyaline pseudopodium is extended, the lipoid granules quickly crowd in behind the hyaline cap. The nuclear cap then moves over and becomes wedged into the base of the pseudopodium, and as soon as the major portion of the cap has swung into the pseudopodium the nucleus and its attached cilium is pulled after it. In making this passage the nuclear cap is not greatly altered in form. After the passage of the nuclear cap and nucleus into the pseudopodium the body of the gamete quickly follows.

Both the male and the female gametes, as has already been noted, are spherical or subglobose in the free swimming condition; both are uniciliate. They are also alike in internal organization so that the above detailed description applies to both male and female gametes. The gametes differ, however, in many salient respects (Fig. 8). First, the female is two to three times as large as the male. Second, the female is grey in colour, the male is of the colour of brass, these being the colours of their lipoid granules. Third, the nuclei in the female are twice as large as those in the

male. Fourth, the volume of the nuclear cap in the female is three to four times as great as in the male. Finally, the male gametes, on the whole, are more active than the female; they become active in the gametangium itself and can be seen moving around jerkily as soon as partial discharge permits.

As might be expected, the general vigour of the gametes differs in different gametangia, and particularly in different cultures. Hence there is no agreement in the duration of their swarming period or in their activity during that period. The duration of the swarming period for females that fail to mate was found to be short, however. In one extreme case the female gametes issued from the gametangium very slowly and they were more than ordinarily delayed in individualizing themselves from the mass in which they escaped. Once free, their cilia moved but weakly and they came to rest almost immediately without conjugating and without leaving the field of a 4 mm. objective. In most cultures the females show vigorous ciliary motion and remain active for a longer period. The male gametes, in the best example that could be held under observation, came to rest in twenty-two minutes, without going out of the field of the 16 mm. objective. Observations on the motion of the gametes are not in complete agreement. When in close confinement, the motion is different from that when free-swimming. When gametangia are hemmed in by hyphae and detritus the male gametes exhibit a darting movement. The gametes dart forward for a short distance and then vibrate, holding approximately the same position; this procedure is then repeated. For the sharp darting movement the cilium, which has swung forward in a loose loop, is snapped back; then, held hard back, it is whipped around in small circles, thus imparting a vibratory or 'wriggling' motion to the gamete itself. The movement of the female gamete when similarly restricted is like that of the male but less rapid.

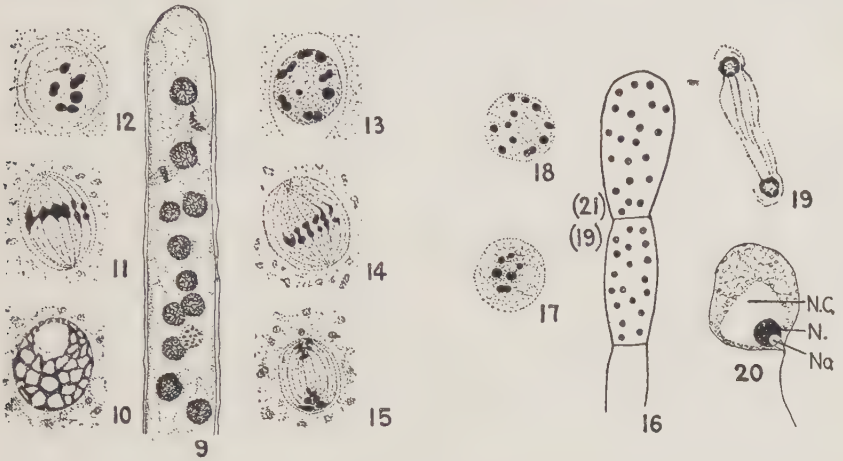
When a gamete is in the open and its movement is unrestricted it glides steadily along; it also apparently turns on its axis as it moves. This is especially evident in a free-swimming zygote, on which a slight bulge marks the position of the male. This irregularity of contour helps to establish the rotary movement of the zyote on its own axis.

The gametes also have the power of amoeboid movement (p. 635); this is particularly marked in the female and is not dependent upon fusion. In certain unfused female gametes which abandoned active ciliary movement and came to rest soon after their discharge, striking amoeboid movement was initiated in about twenty minutes.

IV. Observations on Fixed Material.

The hyphal tips.

Observations on the nature and distribution of the cytoplasm and the cytoplasmic inclusions made with fixed material serve only to confirm those made on living material (see Figs. 21 and 22 for chondriosomes and lipoid granules). They do, however, provide additional information



Figs. 9–15. Nuclear phenomena in the hyphal tip as demonstrated with the Feulgen reaction for chromatin. 9. Hyphal tip showing distribution of nuclei. Three nuclei in mitosis. × 1,200. 10. A resting nucleus. × 3,200. 11. Early anaphase, side view. Chromosomes beginning to split. × 3,200. 12. Early anaphase, polar view. Chromosomes beginning to pull apart × 3,200. 13. Anaphase, polar view. Chromosomes already separated. × 3,200. 14. Anaphase side view. × 3,200. 15. Telophase. × 3,200. Chromosome count six. Compare Figs. 11–15.

Figs. 16–20. Nuclear phenomena in the gametangia as demonstrated with the Feulgen reaction for chromatin 16. Young gametangia but recently cut off from the hypha showing that the nuclear complement of the female gametangium at this time may even be greater than that of the male. × 800. 17. Anaphase, polar view. Chromosomes beginning to pull apart. × 3,200. 18. Anaphase, polar view. Chromosomes separated. × 3,200. 19. Telophase. Spindle stretched and collapsed. Chromosome groups pulling apart. About × 2,000. Chromosome number six, same as in hyphal tip. For counts in gametangia compare Figs. 17, 18, and 19. 20. A female gamete. Chromatic reticulum of the nucleus staining. Nucleolus and nuclear cap unstained. × 1,200. N. = nucleus; NO. = nucleolus; N.C. = nuclear cap.

concerning the nuclei. These, in the terminal segments of the hyphae, are very numerous and are scattered at random in the cytoplasm (Fig. 9). At least one or two of these nuclei are likely to be in mitosis. Only in young reproductive structures is the proportion of the mitosis to the total number of nuclei greater. The chromosome number is six. For mitotic figures and chromosome counts see Figs. 10–15.

Gametogenesis, the 'granular' stage.

When cross walls come in across the hyphal tip the nuclei, being irregularly distributed are segregated to the male and female gametangia in about equal numbers (Fig. 16). The gametangia are thus multinucleate from the very beginning, cross-walls in heavily nucleated hyphae necessarily cut out cells with a fairly full complement of nuclei.

Furthermore, these nuclei when they are first separated from the rest of the nuclei in the hypha are of the same size as the nuclei below them in the hypha. As soon, however, as these two gametangia, so alike in their nuclear constitution, are separated by a cross wall they show a difference in their mitotic behaviour. Although the male gametangium is a smaller structure, and although it is at first fully as well supplied with nuclei as the female, the nuclei of the male undergo approximately twice as many mitoses as those of the female. Nuclear counts were made in male and female gametangia when all mitotic activity had ceased. In one of the couplets selected for this study the male and female gametangia were of the same length. Since, however, the female was ovoid while the male was roughly cylindrical in shape, the volume of the female was somewhat greater. There were nevertheless 23 nuclei in the female, 40 in the male. In other couplets the counts were 12 female to 24 male, 36 female to 67 male (Fig. 5). The number of nuclei differs with the gametangia, but always there is this same approximate two to one ratio. In addition to this numerical difference, and probably in consequence of it, the male nuclei are one-half the size of the female nuclei (Fig. 5). The nuclear-plasmic ratio in the young gametangium is, of course, like that in the hypha, and in spite of the nuclear divisions subsequently occurring in the gametangium it cannot be demonstrated that this ratio itself undergoes any change. The change is in the volume of the individual nuclei. Those in the male lose as much as half their volume, those in the female only a fraction of that. There is, of course, some slight growth in the gametangia as is indicated by the bulging of the lateral walls, and there is a concentration of the cytoplasm. This of itself would allow of some multiplication of nuclei within the ratio.

While these nuclear divisions are in progress the nuclei are scattered at random throughout the gametangia (Fig. 16). These divisions are never simultaneous; only three to four at most will be found in mitosis in the same gametangium at any one time. The precise spacing of the nuclei throughout the gametangia achieved toward the end of the 'granular' stage is in part accomplished during the telophase of these mitoses. It is not at all uncommon to see the chromosome groups at telophase pulling apart for as much as half the diameter of the gametangium (Fig. 19). In this process the stretched spindle can be clearly seen. Chromosome counts made in both male and female gametangia show that the number remains the same as that in the hypha, namely, six (Figs. 17, 18). Thus, the possibility that a reduction division occurs at gametogenesis is eliminated.

The vacuolization of the cytoplasm and the distribution of the lipoid granules and chondriosomes in the 'granular' stage is found in fixed material to be the same as that in living material. Vacuoles are numerous and are widely scattered. Lipoid granules are irregularly and widely

scattered, some few lining up on the nuclear membrane. The chondriosomes consist of longer or shorter filaments, but even the longer filaments are only a quarter of the length they attain in the hypha (cf. Fig. 23 and Figs. 21 and 22). These filaments are scattered through the gametangia and lie in between and around and about the nuclei (Fig. 23).

Gametogenesis, 'gamete origin' stage.

In the 'gamete origin' stage the nuclei, as has been described, migrate peripherally to line up against the gametangial wall. The lipoid granules follow the nuclei to the surface and line up about them, forming a close investment or sheath. In a Champy-Kull preparation (Fig. 24), however, only the skeleton of these lipoid granules stains. Now the chondriosomes also follow the nuclei to the surface and become scattered between them (Fig. 24). Throughout the 'granular' stage the chondriosomes have been fragmenting, so that in the 'gamete origin' stage they take the form of very fine and relatively short filaments.

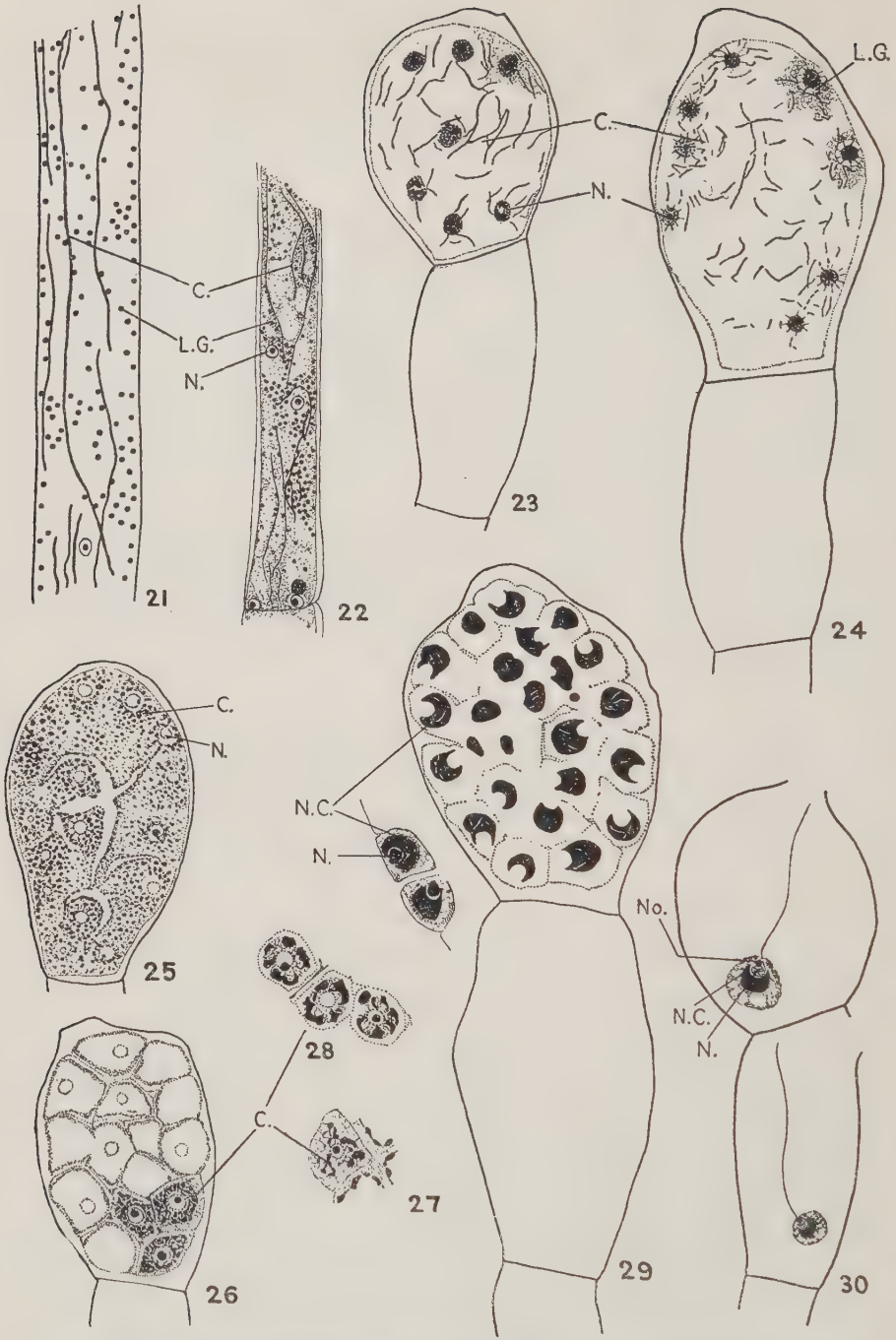
In the further progress of gametogenesis the important feature is the organization of the nuclear cap. This process could not be followed at all adequately in the living condition, so it became necessary to use special chondriosomal technique to follow the process through the critical 'disappearance' stage.

Gametogenesis 'disappearance' stage.

When the nuclei with their sheaths of lipoid granules sink toward the centre of the gametangium the chondriosomes have fragmented and exist only as very short rods or granules; they move with the nuclei but remain peripheral to the sheaths of lipoid granules that still invest the nuclei.

Gametogenesis, cleavage.

As the cleavage furrows push through to the gametangial wall a cloud of chondriosomes is cut off about each nucleus (Fig. 25). With cleavage completed, these minute chondriosomes begin to enlarge (i.e. become vesiculated) and so gain in distinctness (Figs. 26 and 31). Thus each nucleus, probably with most of its lipoid sheath still intact, becomes buried in a mass of chondriosomal granules that must be apparent to even the casual observer. With the further vesiculation of these chondriosomal granules, contiguous chondriosomes coalesce (Fig. 27) to form a reticulum. This chondriosomal reticulum is thrown up about the nucleus in the cytoplasm toward the peripheral limits of the gamete. With further vesiculation the nucleus becomes enveloped in a relatively heavy but ragged mantle (Fig. 28). This mantle now begins to close in on the nucleus. After this manner, then, there is formed one rather large homogeneous mass of indefinite shape and dimensions which, as it invests the nucleus more closely tends to displace the lipoid granules from the nucleus. The final disposition of this



FIGS. 21–30. Cytoplasmic phenomena of gametogenesis as demonstrated with Champy-Kull's chondriosomal technique. 21 and 22. Segments of the hypha. × 800. 21. A segment toward

chondriosomal structure cannot be described satisfactorily until the developments in the rest of the gametangium are understood.

The cleavage furrows, as noted above, first appear in the centre of the gametangium, and gradually work out towards the periphery, cutting out the gametes as they progress (Fig. 25). The gametes in the centre, always relatively few in number, often lie completely separated from their fellows before the peripherally moving furrows have delimited the greater mass of gametes that line the wall. A cross section of a gametangium at this stage would show a single gamete completely delimited in the centre. Cleavage furrows would appear as narrow radiating wedges pushing out to the gametangial wall. Each of the peripheral gametes would appear triangular in a cross section of the gametangium. In three dimensions the shape of one of these peripheral gametes is that of a truncated pyramid with a hexagonal base.

In this process of cleavage the essential thing is apparently that each chondriosomal complex (nuclear cap) should be completely delimited. When the gametes are cut out in a normal gametangium each gamete has but a single cilium, a single nucleus and a single nuclear cap. Under abnormal conditions, however, the cleavage of the cytoplasm may be imperfect. Thus, in the case of one female gametangium three monstrous gametes were discharged along with normal ones. One (Fig. 33) possessed three cilia, three nuclei, and three nuclear caps; the second was roughly of the same size as the first, but it had neither cilia, nuclei, nor nuclear caps; the third was biciliate with two nuclei and two nuclear caps. In a gametangium that produces such monstrosities it is apparent that the process of gamete cleavage has been seriously disturbed. In the three-ciliated gamete the three nuclear caps were joined by their bases; the major portion of each of them, however, stood free of its neighbour. The nuclei stood free as at the apices of three triangles. In the biciliated gamete the same condition obtained: the nuclei were separated, but the nuclear caps joined. Thus it would seem that in the delimitation of gametes the essential thing is that the cleavage furrows should push through and separate contiguous chondriosomal complexes or nuclear caps.

A careful study of any one of the peripheral gametes (Fig. 32) shows

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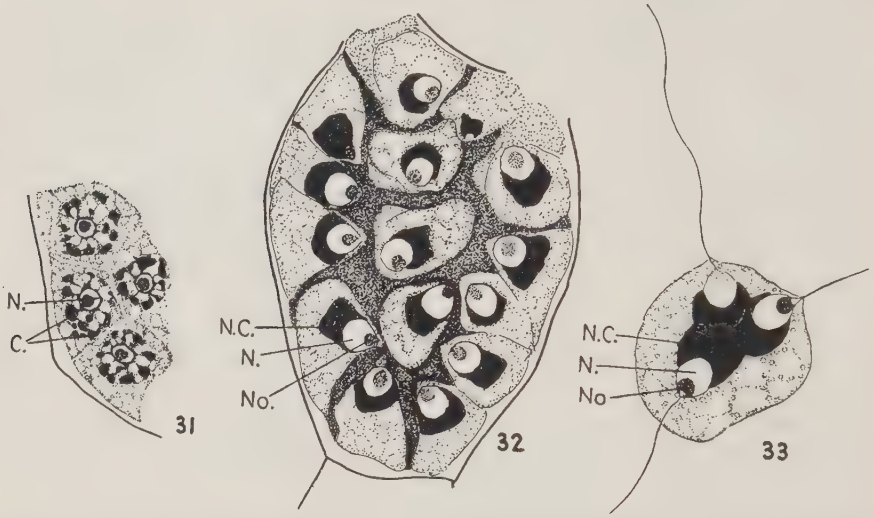
the base. 22. A segment toward the apex. Both show the great length attained by chondriosomes in the hypha. 23. 'Granular' stage of gametogenesis. Nuclei distributed throughout gametangium. Chondriosomes scattered and shorter than those in hyphae. × 1,200. 24. 'Gamete origin' stage. Nuclei displaced peripherally and outlined by the skeletons of lipoid granules. Chondriosomes, short filaments, concentrated peripherally. × 1,200. 25. Female gametangium, 'disappearance' stage. Cleavage furrows formed. Chondriosomes granular. × 1,200. 26. Female gametangium, cleavage completed. Chondriosomes drawn more closely about the nuclei. × 1,200. 27. A gamete from a female gametangium. Chondriosomes enlarging and fusing into a ragged reticulum. × 1,200. 28. Gametes from a female gametangium. Chondriosomal complex organized into a heavy reticulum. × 1,200. 29. Gametangia and insert from female gametangium. Chondriosomal reticulum drawn together into a nuclear cap. × 1,200. 30. Male and female gametes. Nuclear caps staining distinctly. C. = chondriosomes; L.G. = lipoid granule; N. = nucleus; N.C. = nuclear cap.

that the nucleus takes up its position well towards the apex of the pyramidal gamete. The large nuclear cap, the development of which is now being followed, is still applied to the nucleus, but it is external to the nucleus, and lies nearer the wall of the gametangium. Its bulk is pressed



FIGS. 31–33. 31. Insert from a female gametangium. A chondriosomal complex of large granules drawn about the individual nuclei. Gilson, iron-alum haematoxylin. × 1,200. 32. Female gametangium showing the orientation of gametes. Also orientation of nucleus and nuclear cap in individual gametes. × 1,200. 33. Monster with three nuclear caps fused, three nuclei free, and three cilia. From living material. × 1,200. C. = chondriosomes; N. = nucleus; NO. = nucleolus. N.C. = nuclear cap.

into the broadened end of the pyramidal gamete, and takes on the appearance of a heavy crescent-shaped nuclear cap. Apparently, by the crowding of the gametes, the bulky nuclear cap has been forced to develop asymetrically in its later stages so that the nuclear cap takes up its final position to one side of the nucleus, that side where space permits. That a very considerable pressure is exerted on these gametes by their crowded position is evident from the fact that the nucleus and its attendant cap when first formed take the form of their mould; they become definitely pyramidal. Although the nucleus is not completely enveloped by the nuclear cap it is deeply embedded in it.

Most of the lipoid granules in the delimited gametes line up along the cleavage furrows, but there are always some that hug the nucleus and nuclear cap closely. Ultimately the single cilium appears. The cilia of all the peripheral gametes develop at the pointed or inner end of the gamete, and extend into the central region of the gametangium, where there is usually some free space after cleavage has occurred (Fig. 32).

In the formation of this nuclear cap chondriosomes alone are apparently involved. In the living gametangia the lipoid granules can be followed

through gametogenesis and into the gametes. Furthermore, during cleavage when the nuclear cap is being formed toward the centre of the gamete the lipoid granules are concentrated peripherally (Fig. 7). In applying an osmic acid technique to stain the lipoid granules it can be even more convincingly demonstrated that these granules are thrown out into the peripheral cytoplasm, while the nuclear cap is being organized toward the centre of the gametes.

The gametes (Fig. 30).

The study of fixed and stained gametes is instructive in demonstrating the separate identity of the two closely associated structures, nucleus and nuclear cap. For example, the Feulgen reaction for chromatin differentiates clearly between these two structures. While the nucleus proper gives the characteristic pink-to-red reaction the nuclear cap does not stain, but stands out a clear white (Fig. 20). Another critical coloration is that obtained by a combination of Benda fixation and Champy-Kull staining. With this chondriosomal technique, the nuclear cap stains blue, the nucleus red. Even with iron-alum-haematoxylin after either Gilson or Feulgen fixation a clear distinction can be seen between nucleus and nuclear cap.

Observations on the motor apparatus as made on fixed gametes have been incorporated in the description of living gametes.

V. Discussion.

Sex segregation.

In a sexual mycelium each nucleus in the mycelium must be like every other nucleus in its genetic constitution, since all are descendants of the same nucleus, namely, the single nucleus present in the zoospore from which the mycelium arose. From this it follows that in the nuclei of the male and female gametangia at the time when they are delimited from the hypha there can be no difference in genetic constitution. Furthermore, since the nuclei of the hyphae are segregated to male and female gametangia on a random basis the nuclear number is approximately equal (Fig. 16). If then the complement of nuclei in the two gametangia is the same qualitatively and quantitatively at the time when they are delimited from the hypha, when and how do the differences arise that determine that one structure will be male and the other female? Obviously these differences should be traceable to some protoplasmic constituent of the hyphal tip, and obviously to some protoplasmic inclusion other than nuclei. As has been noted before, there are at least two types of clearly defined cytoplasmic inclusions within the hypha: the lipoid granules and the chondriosomes. Since the lipoid granules are rather evenly distributed through the hyphal tip, and are segregated in male and

female gametangia like the nuclei, i.e. in approximately equal numbers, it seems highly improbable that they play any important role in sex segregation. The chondriosomes, however, are unequally distributed through the hyphal tip. In the tip they are crowded (Fig. 1), but are much more diffusely distributed behind. As the cross walls that delimit the gametangia are formed approximately simultaneously, unequal distribution of chondriosomes between the terminal female cell and the subterminal male is likely to result. It is, however, altogether too early to conclude that the chondriosomes are the certain and exclusive agents of sex segregation in *A. arbuscula*.

Sex expression.

In the expression of sexuality, however, the role of the chondriosomes is certainly of primary significance. Between male and female gametangia a quantitative chondriosomal difference is apparent. Between male and female gametes the disparity in amount of chondriosomal material is still more apparent. The chondriosomal nuclear cap of the female is always three to four times as large as that of the male (Figs. 8 and 30). In the chondriosomes, then, we have a definite quantitative index of sexuality, i.e. of maleness and femaleness.

Another distinction between the sexes in *A. arbuscula*, quite as remarkable as this differential distribution of chondriosomes, is the difference in colour between male and female gametangia and male and female gametes. These distinctions depend on the different colours of the lipoid granules in the two cells. Since the brilliant male colouring does not develop until rather late in gametogenesis when the male gamete origins have become rather well organized and the maleness of the gametangium has become relatively well established, it would seem that these cytoplasmic inclusions play no part in sex segregation. Their ultimate pigmentation is, however, a striking qualitative expression of maleness, their lack of pigmentation, of femaleness.

The size of nuclei in gametangium (Fig. 5) and gamete (Fig. 8) is another index of maleness and femaleness in that the male nuclei are but half the size of the female nuclei. This contradistinction, however, seems almost certainly to be the expression of a nuclear-plasma ratio than an expression of sexuality itself. The direct expression of sexuality in this particular is the differential mitotic potentialities developed in the two gametangia. The nuclei were segregated to male and female gametangia in approximately equal numbers. Why the nuclei in a smaller cell should later undergo roughly twice as many mitoses as the nuclei in a larger cell is difficult to explain. The most likely explanation is that there is a fundamental difference in the cytoplasm of male and female gametangia, which difference is capable of affecting the division rate of the nuclei.

The nuclear cap.

Ever since 1878 when Reinsch (14) first discovered *Blastocladia*, students of the water moulds have been puzzled by the peculiar type of nucleus found in the zoospores of this genus. Since 1911 when Butler (3) discovered *A. arbuscula*, other investigators have been interested in the nuclei of the zoospores of *Allomyces*, because these show the same peculiarities as those of the zoospores of *Blastocladia.* Despite the interest shown in these nuclei, no adequate explanation of their singular structure has yet been given; nor has there been any consistent progress in our knowledge of their structure. Barrett (2) in 1912 interpreted these nuclei correctly, concluding that the major portion of the 'large, subtriangular, centrally located body' in the zoospores of *A. arbuscula* (his *Blastocladia strangulata*) was a 'food body', the nucleus being simply imbedded in this 'food body'. Kniep (11) 1929 accepted Barrett's interpretation for *A. javanicus*, but Cotner (6) 1930, working on Thaxter's *B. pringsheimii*, turns back to Thaxter's (16) original misconception that the 'very large and subtriangular' body was all nucleus.

Although the present paper treats of gametes rather than of zoospores it nevertheless presents evidence bearing on this problem of the organization of the 'subtriangular' body in the zoospores of the Blastocladiaceae. In *A. arbuscula* gamete and zoospore are alike in possessing a nuclear cap, which has been shown to be extranuclear. It has been demonstrated, further, that this extranuclear body or cap is formed from chondriosomes. It can, therefore, be stated with confidence that Barrett's interpretation of the nuclear cap (his 'food body') was certainly correct for the zoospores of *A. arbuscula* and, judging from Thaxter's and Cotner's descriptions and figures, it seems certain that the same explanation holds for the 'very large subtriangular' body described by them in the zoospores of *Blastocladia pringsheimii.*

The discovery of a formed body of chondriosomal origin (the nuclear cap) (Fig. 8, 20, 29, 30, 32, and 33) in the gametes and zoospores of *A. arbuscula* is interesting in a limited sense because it explains the structure of certain anomalous nuclei in the zoospores of the Blastocladiaceae. It is interesting in a larger sense because it is apparently the first time that such a structure has been described in the motile cells of plants.

Although a search of the literature on zoosporogenesis, gametogenesis, and spermatogenesis reveals no strictly analogous structure there is in the spermatozoids of mosses a bulky cytoplasmic inclusion that may prove to be the equivalent of this nuclear cap. This structure, in the mosses is known as a limosphere. The term limosphere was first used by M. Wilson (19) in 1911 in describing a cytoplasmic inclusion that he had noted in the spermatids of certain mosses. Allen (1) in his investigation of spermatogenensis

in *Polytrichum juniperinum* was the first to make a careful study of this structure. The limosphere, according to Allen, first makes its appearance in the young spermatid (androcyte) as a spherical, evenly staining mass. This gives off an apical body which becomes applied to the anterior end of the nucleus. The major part, or limosphere proper, temporarily becomes lodged against the posterior end of the nucleus. Later the limosphere remnant disappears from the spermatozoid. The derivation of this limosphere from plastids was first demonstrated by Weier (17) who showed that the 'kinoplasmic plates' of Allen, which appeared in the early spermatogenous cells (androgones) but which disappeared from the androgones before their last mitosis, were actually plastids as had been earlier suggested by Sapehin (15). Weier was not only able to trace the 'kinoplasmic plates' back to the plastids in the young antheridia, but he was able to follow them through the androgonial divisions and into the androcytes where they gave rise to the limosphere. Motte's (13) description of the origin of the limosphere is somewhat different from Weier's. Motte believes that the plastids in the androgonial cells fragment, and then, in the androcyte, join with the chondriosomes already present in the cell to form a granular mass which condenses to form the solid limosphere.

In *Allomyces* it can be definitely shown that the nuclear cap is formed from a complex of chondriosomes. Whether the limosphere of *Polytrichum* is formed from a chondriosomal complex or a plastid complex seems to be only a matter of interpretation. Motte, as we have seen, believes that the limosphere may be formed of chondriosomes, at least in part, and Weier's figures of his plastid complex, as he traces it through the androgonial divisions and into the androcyte, certainly suggest that it may be just as well considered as a chondriosomal complex. His 'granular plastids' and his plastids that look like 'a delicate chromatic reticulum embedded in an achromatic matrix' certainly appear more like the accepted picture of a chondriosomal complex. Judging from Weier's figures and my slides, I believe that the limosphere in *Polytrichum* is built up in quite the same manner as the nuclear cap in *Allomyces*; that is, the chondriosomes first aggregate (Fig. 25), then vesiculate (Fig. 27), and finally fuse (Figs. 27–9). In the process of fusion however, they first form a fine reticulum (Fig. 27), later, a heavier reticulum (Fig. 28), and ultimately a single mass (Fig. 29). Thus it would appear that the origin of the limosphere and the nuclear cap, if not exactly the same, is yet very similar.

It has been suggested (Weier, 17) that there is a close similarity between the limosphere of *Polytrichum* and the acroblast of certain animal sperms. In the case of *Allomyces*, however, if any analogy is to be drawn between the nuclear cap in gamete and zoospore and any structure in the animal sperm it apparently must be with the 'nebenkern'. Here the

analogy seems to be very close. In the sperms of certain Diptera, for example, it has been shown that after the last spermatogonial division, as the interzonal fibres are pinched in two, the chondriosomes that were arranged along the sides of the spindle are pinched apart. The chondriosomes are thereby divided roughly into two equal groups and are left clumped in the interzonal region of each spermatocyte. In this chondriosomal aggregate the individual chondriosomes come to lie more or less parallel to each other, and by a process of progressive vesiculation fuse along their long axes to form a single large mass, the nebenkern.

The developmental history of nebenkern and nuclear cap run parallel with each other most remarkably: (1) both structures are formed out of a chondriosomal complex, (2) in both this complex arises from the vesiculation of individual chondriosomes, and (3) in both the structures form a single large mass.

VI. Summary.

1. In the hyphal tips of *A. arbuscula* there are numerous nuclei, lipoid granules, and chondriosomes. Nuclei and lipoid granules are distributed at random; the chondriosomes are concentrated in the tip. The nuclei have six chromosomes; the lipoid granules are grey-black in colour, small and spherical; the chondriosomes are long and filamentous.

2. When the male and female gametangia are cut off from a hyphal tip the nuclei and lipoid granules are distributed between the male and female gametangium in approximately equal numbers. The chondriosomes segregate unequally, a disproportionately large number going to the terminal, female gametangium.

3. In early gametogenesis the nuclei divide more often in the male gametangium, so that it contains roughly twice as many nuclei as the female. These male nuclei are one-half the size of the female nuclei. The chromosome count, however, is six in the nuclei of both gametangia. These nuclei become the 'organization centres' in the formation of gametes, and each collects about itself a sheath of lipoid granules and a tangled mass of fine, filamentous chondriosomes.

4. In late gametogenesis the lipoid granules in the male gametangium change colour, becoming salmon-pink, while those in the female remain grey. The chondriosomes fragment into a cloud of small granules, and these granules subsequently enlarge and fuse to form, first, a reticulate mantle about the nucleus, and finally, a single large mass which becomes appressed to the nucleus in the form of a nuclear cap.

5. The female gamete is two to three times as large as the male; its nucleus is twice, and its nuclear cap three to four times, as large as that of the male. Its lipoid granules are grey, while those of the male are of a brassy colour.

6. Since the nuclei in the hyphae are genetically the same and are segregated in male and female gametangia in equal number, there appears to be here a peculiar type of differentiation in which the distribution of chondriosomes is of primary importance in the determination of sex.

7. In the visible expression of sexual differences there is a definite quantitative factor in the amount of chondriosomes (nuclear cap) and a qualitative factor in the colour of the lipoid granules.

8. The extranuclear nature of the nuclear cap found in the gametes and zoospores of *A. arbuscula* is demonstrated and its chondriosomal origin described. An analogy is suggested between this nuclear cap and the limosphere found in the spermatozoids of mosses. In animals the nebenkern of certain spermatozoa (Diptera) suggest even closer analogies.

To Dr. Duncan S. Johnson under whose direction this research was carried out, I wish to make acknowledgement of his stimulating and helpful criticism; to Dr. John N. Couch, who from a distance has guided and directed this study, I also wish to make acknowledgement; and to Mrs. Helen R. Rosenfeld, Miss Elizabeth H. Gay and Dr. William L. Doyle, who have all actively interested themselves in this research, I wish to express my gratitude.

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VITA

WINSLOW ROPER HATCH

Born, Lexington, Massachusetts, 1 March, 1908

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